HPIC ANALYSIS OF PHOSPHITE AND PHOSPHATE EXTRACTED FROM PLANT SAMPLES

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INTRODUCTION

Diseases caused by members of the genus *Phytophthora* have, for a long time, been recognised globally as among the most devastating plant diseases (1). In Australia, diseases caused by *Phytophthora* spp. cause widespread losses in pastoral, agricultural, horticultural, ornamental and forestry industries, and cause loss of biodiversity in national parks and nature reserves throughout the country (1).

The chemical control of *Phytophthora* diseases in Australia primarily involves systemic fungicides based on phosphonic acid; the active constituent being the phosphonate (phosphite) anion (2). The use of phosphite fungicides has had a significant impact on disease control within Australia (1). Although the mechansim of action of phosphite remains to be clarified, its effectiveness to inhibit growth and sporulation of pathogenic agents motivates the search for a method of determining this anion in biological systems (3).

In the past, analysis has been achieved by Paper Chromatography (PC) (4), Gas Chromatography (GC) (5, 6) and High Performance Ion Chromatography (HPIC) (7, 8, 9, 10, 11). Most procedures are tedious and costly, HPIC columns degraded rapidly and in many cases are no longer available. The advent of new HPIC resin columns was heralded as the answer to the problamatic analysis of biological phosphite extracts, however the current investigation proved this not to be the case.

The current investigation, which is based on previous research (7), offers a robust, rapid and long term analytical method for the analysis of biological samples which contain both phosphite and phosphate.

MATERIALS AND METHODS

Plant material 0.5g (dry weight) of finely ground plant tissue was shaken and extracted (over night) into a 5mL aliquot of double de-ionised water. Enough extract to obtain 200uL was filtered through a 0.45µm nylon acrodisc (Gelmann Sciences).

Chemicals Phosphite and phosphate standards were prepared from Phosphorous acid (99%) (Aldrich) and potassium phosphate mono-basic (99%) (Baker), respectively. Mobile phase was prepared from succinic acid (99+%) (Aldrich) and pH adjusted with lithium hydroxide monohydrate (Sigma).

Phosphite and phosphate analysis The ion chromatographic system comprised a Waters 501 HPLC pump with a Waters 712 WISP auto injector and an Alltech 320 conductivity detector with sensitivity 0.01 uS full scale and positive polarity. The chromatographic data was recorded and processed using Millennium Chromatography Software version 2.15.01. 50µL sample injections were separated using a Vydac 302IC4.6 (0.46 x 25cm) silica based non-supressed ion chromatography column, with 20mM succinic acid (pH3.5) mobile phase flowing a 1mL/min. The mobile phase was filtered through a 0.45µm nylon membrane and degassed with helium. Samples were analysed within 24h. to prevent microbial growth. Column regeneration was performed every 24h. according to the following 1) 0.5% nitric acid (300mL), 2) water flush, 3) methanol (100mL), 4) water flush and 5) re-equilibrate with succinic acid mobile phase.

RESULTS AND DISCUSSION

The governing factors for the analysis were 1) the pH dependant speciation of phosphite and phosphate and 2) the pH operating ranges of the available HPIC columns. It was determined through exhaustive testing that pH3.5 offered both a single species of the phosphite and phosphate analyte anions at a concentration maximum and the optimal operational pH of the Vydac 302IC4.6 column. The investigation determined a) an over night period was required for analyte extraction, b) phosphite did not converted to phosphate within plant tissue and c) complete extraction of phosphite was achieved. It was also determined that water, used as an extracting solvent in place of succinic acd, achieved superior analyte extraction and reduced baseline drift in the conductivity detector.

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